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Studies on Method of Infection and Control of the Red Rot Fungus of Sugarcane.

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STUDIES ON METHOD OF INFECTION AND CONTROL OF THE RED ROT FUNGUS OF SUGARCANE

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ABSTRACT

Information leading to a better understanding of the method of infection of the sugarcane stalk by the red rot fungus, Physalospora tucumanensis Speg., should aid in the development of control measures for the disease. Steib (40) reported that the fungus occurred in the stalk in a latent form and found that this latent infection developed in stalks placed under unfavorable conditions, infection occurring at the nodal region. The initial points of entry into the stalk were the leaf scar and the bud.

Results of these studies demonstrated that the red rot fungus could not be eliminated from the leaf scars, buds, and bud scales of variety Co. 290 by soaking for 24 hours in a solution of bichloride of mercury. Stalks of different varieties placed in storage developed red rot, infection occurring at the node. With all varieties tested, the initial points of infection could be traced to the leaf scar and bud. A correlation was found between resistance to the disease and development of the organism in the stalk. Of several fungicides used to treat the cane before storage with the fungicide residue remaining on the cane, Puratized N5E was the only one which gave a reduction in the number of nodes developing red rot. Removal of the leaf sheaths from stalks of Co. 290 before infection by the fungus greatly reduced the amount of latent infection in the nodal region. Occurrence of the conidial stage of the organism on the leaf sheaths was the same for resistant and susceptible varieties. The conidial and perithecial stages of the fungus were found on a high percentage of mature leaf sheaths and leaves of variety Co. 290.

Studies on the nature of bud infection showed that penetration of the epidermis of young bud scales by infection threads occurred 33 hours after inoculation with a conidial suspension of the red rot fungus. A cap or overgrowth around the infection thread was found in both a resistant and susceptible variety. In the resistant variety studied, the cap was seen in epidermal and subepidermal cells. Mycelium was observed in cells below the epidermis only in cases in which the epidermal cells were thin-walled. A dark gummy material often filled the intercellular spaces below the point of infection. This material was produced in lesser amounts in the susceptible variety. Young shoots became infected at the point of contact with the old bud scales. Red rot developed in stalks placed under dry conditions, but failed to develop when moist conditions prevailed.

A fungicidal dust applied on the cane during the growing season reduced the amount of latent infection in the stalk. Certain planting tests in which dusted cane was used for seed did not give increases in yield, due to the deterioration of the seed pieces by Phytophthora. However, tests conducted on two plantations gave increases in shoot count in the spring. A planting test in which dusted cane was treated with a fungicide before planting gave significant increases in shoot count and yield. Dusting Co. 290 cane to be used for seed with either Cryolite or a combination of Cryolite and Parzate in an area in which the sugarcane borer was a problem gave an increase in stand in the spring. Stand was improved with Parzate dusted cane only in tests which were planted on heavy soil. Early planting of Co. 290 was found to be better than late planting.

Results obtained in these studies indicate that the sugarcane stalk becomes infected with the red rot organism sometimes during the growing season and that this infection remains in a latent form until dry conditions or

other environmental factors reduce the vitality of the stalk, allowing further invasion by the organism.

INTRODUCTION

Red rot of sugarcane caused by Physalospora tucumanensis Speg. is considered one of the most important diseases of sugarcane in Louisiana. It had been under investigation in Louisiana since it was first found in this state in 1908 (18). This disease, unlike the other two major diseases of sugarcane (mosaic and root rot) in Louisiana, causes a reduction in the number of shoots emerging from the mother stalk in the spring. Any information leading to a better understanding of the deterioration of the cane during the winter months should be of value in the development of control measures which would stabilize the stand of cane in the spring.

The part played by red rot in reducing stands is recognized by both the plant breeder and plant pathologist. New varieties of sugarcane are not released unless they show some resistance to the disease in inoculation tests.

An understanding of the method of infection and development of the disease in the stalk is related to the problem of red rot control. A study of host-parasite relations might furnish information on the mode of penetration and nature of resistance to the disease. More information on the time at which infection of the stalk takes place might help in the development of control measures.

The use of seed treatments in the past in an attempt to stabilize stands in the spring have given erratic results. A study of the mechanism of infection and time at which latent infection of the bud scales and leaf scar occurs should help explain the results obtained with seed treatments.

Such information should also lead to the development of some other control measure such as the use of a protective dust on seed plots during the growing season.

The studies included in this thesis were begun in order to obtain information concerning the mode of infection of the sugarcane stalk by the red rot fungus. The results obtained, relative to the mode of infection, led to a study of control measures for the disease. This included the use of a fungicidal dust on the cane during the growing season and the use of dusted seed cane in combination with seed treatment to increase the yield of the very susceptible variety of sugarcane Co. 290.

HISTORICAL REVIEW

The fungus causing red rot of sugarcane, Physalospora tucumanensis Speg., most commonly known as Colletotrichum falcatum Went, was first described in Java by Went (44) in 1893. The disease has now been reported from all the major sugar-producing countries of the world. According to Butler (10), it caused severe losses in Bengal in 1906. In 1908, Edgerton (18), working in the United States, reported the deterioration of seed cane in Louisiana and Georgia as due to the disease. Red rot caused great losses in Louisiana in 1923, according to Edgerton, Taggart and Tims (24). The next year, the year of the first severe cane failure in Louisiana, 40 to 50 per cent of the young cane plants died as a result of the disease (24). It was reported by Edgerton and Moreland (23) that germination of the eyes was reduced by nearly 50 per cent when seed cane was inoculated with spores of the fungus.

Sugarcane stalks affected with the disease have a lower per cent sucrose. According to numerous investigators (2, 9, 10, 18, 20, 21), the fungus causes an inversion of sucrose to dextrose and levulose.

There are conflicting reports in the literature in regard to the mode of entry of the red rot organism into the sugarcane stalk. Went (44) concluded that natural infection occurred chiefly through holes made by boring insects. Workers in India, however, found little or no natural infection through borer holes (10). That borer holes were the principal means of natural infection of the stalk was also reported by Lewton-Brain (32), Edgerton (21), and, according to Abbott (3), by workers in the West Indies.

Abbott (3) reported that Raciborski (35) described the spread of the fungus from the seed piece to the growing stalk, and this was confirmed in India (10, 11), but not in Louisiana (3, 21) or the West Indies (39).

Other points of entry of the organism into the stalk have been reported. The root band region (11, 3, 33), leaf scar (19, 29), bud (11), mechanical wounds, and growth cracks have been suggested. Butler and Hafiz Kahn (11) did not consider the leaf scar as an important point of entrance. They stated that since the leaf scars are normally not exposed until the leaf has completely withered, penetration by the fungus was impossible. Steib (40) stated that infection could take place through the leaf scar. However, he showed that the leaf scar became infected during the growing season before the leaf had completely withered. Howard (29) obtained infection by inoculation of the leaf sheath base. Nesom (33) reported that the organism migrated from one part of the leaf to the other through the ligular region, but no such migration probably took place by means of spores which were carried through the vascular bundles in the transpiration stream. Earlier, Atkinson (4) and Atkinson and Edgerton (5) demonstrated that spores of the fungus could migrate through the stalk by way of the vascular bundles.

Edgerton (19), after examining diseased stalks obtained from Georgia, expressed the opinion that infection must have taken place at the nodes, since no burrows made by borers were found in stalks showing symptoms of the disease. Recent work by Steib (40) showed that infection can occur at the nodal region independent of borer holes. Johnston and Stevenson (30) of Porto Rico found that the disease occurred to a limited extent independently of borer holes, but it was generally possible in such instances to find some other weakening influence, such as drought, root disease, or other fungi.

No mycelial connection was found between the seed piece and the young plant by Edgerton (19) and Abbott (2) in Louisiana. Work done in the West Indies (39) cited by Abbott (3) agreed with conclusions in Louisiana. Butler (10), of India, disagreed and stated that most of the growing plants became infected in this manner. Edgerton and Moreland (23) stated that conditions in India were radically different from those in the West Indies and Southern United States. Just why the fungus acted differently in the different countries was not known. It may be that there were different strains of the fungus in the various countries or that there were differences in varietal susceptibility or climatic conditions.

Edgerton and Moreland (23) pointed out that there were small thin places in the rind of the nodes where the young roots emerged and postulated that through these points the fungus gained entrance to the seed cane. Butler and Hafiz Khan (11) were the first to suggest this as a possible mode of entry. Abbott (3) conducted experiments in which the root band regions were smeared with agar cultures of the organism. Sterilized cuttings of one variety showed no evidence of infection through the root region while another variety showed 81 per cent infection. Steib (40) was able to isolate the organism from the root region even after 24 hours immersion in a solution of bichloride of mercury. However, after examination of surface-sterilized canes which were placed under suitable conditions for development of the disease, he concluded that the initial point of entry was through the bud and leaf scar and not the root band region.

Laboratory experiments indicated to Abbott (3) that the fungus could enter the stalk through the cut ends. Upon examination in the field it was found that such infection was small. Edgerton and Moreland (23) pointed out that yeasts and other organisms invaded the cut ends, and that these

served to prevent the growth of the fungus.

Butler (10) found the fungus on young shoots shortly after germination. Evidence indicated that the fungus was not carried to the young shoots through the air but was either present previously in the soil or was carried on the setts themselves. Abbott (1, 3) failed to isolate the organism from the soil and also stated that seed cane in Louisiana was commonly planted with leaves and sheaths adhereing to the stalk. This meant that an abundant supply of red rot spores and mycelium went into the soil with seed cuttings, in addition to that which already may have established itself within the stalk and in the soil. Further infection might then take place from this inoculum through borer holes or through the nodes. A recent report from India by Dastur (13) stated that experiments have demonstrated the transmissibility of infection on a considerable scale through contaminated soil and irrigation water with the nodal regions of the cane plant indicated as the most susceptible to attack. The fungus was found to survive for about six months in fallow land. Shepherd's opinion (37) was that when an entire stool of cane showed symptoms of the disease, it could be inferred that infection of the subterranean portions of the stool had been accomplished by spores which were blown to the soil from the fructifications of the fungus on affected stems or leaves or both, or which were set free in the soil after the rotting of diseased parts of the cane in the field, or that the stool originated from an infected cutting. Padwick in India (34), after studying the possible modes of entry of the fungus into the stalk concluded that the stalk may become infected, by the fungus growing from the mother stalk into the shoot, through borer holes, root primordia and leaf scars, cut ends of seed pieces and miscellaneous injuries. Ghona and Padwick (12) gave results of field experiments which

indicated that diseased cane debris and fungus mycelium added to soil which was later planted to cane, could serve to spread the fungus to healthy shoots.

The presence of the red rot fungus in a dormant or latent form in the sugarcane stalk was first suggested by Steib (40). Evidence was presented which showed that the organism could be isolated from the nodal tissues of the cane after severe periods of surface-sterilization.

Shear and Wood (36) were among the first to call attention to the occurrence of dormant infections on leaves and shoots of orange, pomelo, lemon and mandarin. The pathogen, Colletotrichum gloeosporioides, was isolated from these after having been immersed for from 5 to 15 minutes in a 0.2 or 0.1 per cent solution of corrosive sublimate. Bates (8) in 1936, reported that C. gloeosporioides formed dormant infections in oranges. He confirmed this report by isolating the organism from small pieces of skin taken from surface-sterilized fruit. The same worker showed that Alternaria citri could form latent infections in the button and outer rind tissue. Baker and Wardlaw (7) also used the isolation technique to determine the presence of fungi within the rind tissue of mature and immature grape fruit. Latent infection in the banana was demonstrated by Dastur (14) who showed that the development of anthracnose (Gloeosporium musarum) on the plantain could not be prevented by surface-sterilizing the fruit with copper sulphate, formalin, or corrosive sublimate. He also proved that anthracnose could be prevented by field spraying only if the fungicide was present on the fruit from the time the bunch was first thrown.

Wager (42), in South Africa, found that surface disinfection of unblemished mango fruits did not prevent the subsequent development of ripe rot and suggested that infection took place in the green stage. By

isolation techniques, Baker and Wardlaw (7) showed that latent infections evidently existed in the fruits from an early age. In a later paper, Baker (6) listed Colletotrichum gloeosporioides, Guignardia sp., and Phomopsis citri as being the organisms responsible for these dormant infections. Wardlaw, Leonard and Baker (43) found that the papaw was also subject to the same type of infection. Baker (6) also applied the isolation procedure to the avocado, tomato and cacao. On each of these hosts evidence was obtained for latent infection by C. gloeosporioides and Guignardia sp.

Simmonds (38) stated that there are two well-defined forms of latent infection in fruit. In one case the germ tube of the fungus concerned enters a stoma and the mycelium remains in the stomatal cavity, free from the effects of any external application of fungicides, until such time as ripening of the fruit or other factors provide conditions suitable for further invasion. In the second form of latent infection, the fungus penetrates the cuticle directly, and maintains a dormant existence in the superifical tissues until such time as further activity is permitted. Simmonds (38) found by histological technique that the latter was true for anthracnose of banana. Fulton (26) also found that the fungus which causes tomato anthracnose (Colletotrichum phomoides) remained latent between the epidermal cell wall and cutin layer, and that it developed as the fruit ripened.

According to Simmonds (38), the fungi most commonly implicated in the latent type of infection belong to one or the other of the genera Gloeosporium and Colletotrichum. It is typical of the members of these genera to form appressoria at the time of spore germination, and these organs apparently play an important part in the act of infection leading to the latent infection.

Infection by means of the appressorium was first investigated by Hasselbring (28) with Gloeosporium fructigenum on Berberis Thumbergii and the apple, by Leach (31) with Colletotrichum lindemuthianum on the bean, by Gardner (27) with C. lagenarium on cucurbits, and by Dey (15) with C. gloeosporioides on citrus. Edgerton and Carvajal (22), working on host-parasite relations of Physalospora tucumanensis, found that spores introduced behind actively growing leaf sheaths produced appressoria and infection threads. After growing for a period in the host tissue, fruiting structures with spores were produced. Abbott (3) and Steib (40) stated that spores produced on the leaf sheath could serve as a source of inoculum throughout the growing season.

MATERIALS AND METHODS

The sugarcane varieties used in these red rot studies included both susceptible and resistant varieties. Variety Co. 290, susceptible to red rot, was used in most of the work. Other varieties used were: C.P. 34/120 (susceptible), C.P. 36/106 (resistant), C.P. 29/320 (susceptible), C.P. 29/120 (resistant), C.P. 29/116 (resistant, C.P. 36/13 (resistant, C.P. 36/19 (resistant, C.P. 36/183 (resistant, and D-74 (susceptible.

Oatmeal agar was used as the culture medium. It was made by using 65 grams of oatmeal and 20 grams of bacto-agar per liter of water. The oatmeal was placed in a liter flask containing 500 cc. of water and hot water (65° C.) was allowed to run over the flask for 30 minutes to 1 hour. This was then strained through cheese cloth. The agar was mixed in 500 cc. of water and the resultant solution brought to a boil. The oatmeal extract and water agar were thoroughly mixed by pouring back and forth between two pots. The medium was sterilized by autoclaving for 1 hour at 17 pounds pressure. One drop of 50 per cent lactic acid was added to each plate to acidify the agar and reduce the bacterial contamination.

In plating tests to determine the presence of the red rot fungus, the stalks were immersed in a 1-1000 solution of mercury for 10 minutes. After sterilization, the cane was placed in a saturated solution of calcium hypochlorite before plating. The sterilizing agent was made by dissolving two tablets of bichloride of mercury (Parke, Davis and Company) in a liter of solution made up of 500 cc. of 95 per cent ethyl alcohol and 500 cc. of water.

The bud, bud scales and leaf scar were the tissues plated subsequent to sterilization. A pair of sterile forceps was used to remove the two outside scales which cover the bud. These were plated separately from the bud. The bud was removed with a metal inoculator, an instrument similar to a cork borer. The inoculator was sterilized by dipping in alcohol and flaming. The leaf scar was cut out with a sterile scalpel and was placed in the petri dish by the use of sterile forceps.

Stalks of different varieties were immersed for 5 minutes in different fungicides. Varieties Co. 290 and C.P. 34/120 also were treated for 30 minutes in a 1-1000 solution of bichloride of mercury. After treatment, the canes were placed in storage with the fungicide residue remaining on the cane. Subsequent to storage for 30 days at 70°F., the leaf scars and buds were removed with a knife in order to study the effect of the different fungicides on development of red rot in the stalk. The following fungicides were used in the studies:

1. 5 per cent Puratized N5E (10 per cent phenyl mercuri triethanol ammonium lactate) at 1-500.
2. Phygon (Dichloronaphthoquinone) 1.0 per cent suspension.
3. Spergon (Tetrachlorobenzoquinone) 1.0 per cent suspension.
4. Tersan (Tetramethyl thiuram disulfide) 1.0 per cent suspension.

Young bud scales used in penetration studies were inoculated with a spore suspension of the red rot fungus and sectioned, using the following technique. After 33, 50, 60 and 77 hours incubation in a moist chamber, the scales were removed from the bud and then placed in pith and clamped tightly in a screw clamp. Then, by holding the clamp with the left hand under a dissecting microscope, small slices of tissue were sectioned with

a sharp razor blade and transfered by means of a transfer needle to a glass slide on which was placed a drop of lacto-phenol containing cotton blue. Microscopic studies were made 3 or 4 hours after the sections were placed in the stain.

EXPERIMENTAL RESULTS

Effect of Different Periods of Sterilization on Isolation of the Red Rot Fungus

In order to determine the effect of different periods of surface sterilization upon isolation of the red rot organism from the leaf scars, buds and bud scales of sugarcane, apparently healthy stalks of Co. 290 were immersed in a 1-1000 solution of bichloride of mercury in 50 per cent alcohol for periods varying from 10 minutes to 24 hours. The treated stalks were placed in a saturated solution of calcium hypochlorite before plating. The bud scales, buds and leaf scars were then plated on oatmeal agar. The results of the isolations are summarized in Table I.

The fungus was isolated from leaf scars, buds and bud scales after 24 hours immersion in a bichloride of mercury solution. Of 355 nodes plated in the various treatments, the red rot fungus was isolated from 49.3 per cent of the leaf scars, 5.4 per cent of the buds and 32.4 per cent of the bud scales. There was a gradual reduction in the number of isolations from the different tissues as the severity of the treatment increased. Nodal tissues treated for periods of 30 minutes or longer gave pure cultures of the organism.

Table I. Number of Leaf Scars, Buds and Bud Scales of Sugarcane, Variety Co. 290, Giving *Physalospora tucumanensis* after Various Periods of Sterilization in 1-1000 $HgCl_2$ Solution in 50 Per Cent Alcohol Followed by a Saturated Solution of Calcium Hypochlorite.

	Time in 1-1000 $HgCl_2$ solution in 50 per cent alcohol before plating				
	10 min.	30 min.	12 hrs.	24 hours.	Total
Number nodes	55	100	100	100	355
Leaf scars giving the red rot fungus	33	73	48	21	175
Buds giving the red rot fungus	8	4	4	3	19
Bud scales giving the red rot fungus	38	71	5	1	115
Per cent leaf scars giving the red rot fungus	60.0	73.0	48.0	21.0	49.3
Per cent buds giving the red rot fungus	14.5	4.0	4.0	3.0	5.4
Per cent bud scales giving the red rot fungus	69.1	71.0	5.0	1.0	32.4

Occurrence of the Red Rot Fungus in Leaf Scars, Buds and Bud Scales of Different Varieties

Results of previous tests indicated that the red rot organism was present in a high percentage of the leaf scars, buds and bud scales of Co. 290 cane. This test was made to obtain information on the occurrence of the fungus in the nodal region of other commercial varieties. All varieties used were sterilized with a solution of bichloride of mercury for a period of 10 minutes, followed by a saturated solution of calcium hypochlorite before plating. The varieties used and results of isolations are given in Table II.

Table II. Isolation of Physalospora tucumanensis from Different Varieties of Sugarcane.

Variety	Nodes plated Number	Leaf scars giving the fungus Number	Leaf scars giving the fungus Per cent	Buds giving the fungus Number	Buds giving the fungus Per cent	Bud scales giving the fungus Number	Bud scales giving the fungus Per cent
D-74	100	46	46.0	2	2.0	47	47.0
Co. 290	165	38	23.0	9	5.5	43	26.1
C.P. 34/120	214	38	17.6	7	3.3	65	30.4
C.P. 29/320	100	34	34.0	14	14.0	59	59.0
C.P. 36/105	190	28	14.7	2	1.1	41	21.6
C.P. 29/116	50	0	0.0	0	0.0	0	0.0
C.P. 36/19	50	1	2.0	0	0.0	1	2.0

The four varieties giving the highest number of isolates were: D-74, Co. 290, C.P. 34/120 and C.P. 29/320. These varieties, according to field inoculation tests, are susceptible to red rot. C.P. 36/105, which is resistant, had a much higher percentage of infected nodes than the other two resistant varieties plated. Of 50 nodes of C.P. 29/116, none gave the organism. Of the 50 nodes of C.P. 36/19, the fungus was isolated from only one leaf scar and one bud scale.

Results of Isolation from Five Plantations

In order to have some information on the occurrence of Physalospora tucumanensis in the sugarcane area of Louisiana, canes of varieties Co. 290, C.P. 34/120 and C.P. 36/183 from various plantations were brought to the laboratory and plated. The leaf scars, buds and bud scales were plated after 10 minutes in a solution of bichloride mercury followed by calcium hypochlorite. Stalks of Co. 290 which had been dusted with Cryolite in the

field were also plated. This was done to determine whether or not field dusting with Cryolite reduced the amount of latent infection in the stalk. The results are summarized in Table III.

Table III. Occurrence of the Red Rot Fungus in Leaf Scars, Buds and Bud Scales of Three Varieties Collected from Different Locations.

Location	Variety	Nodes plated Number	Nodes giving the fungus from Leaf scar Number	Leaf scar Per cent	Bud Number	Bud Per cent	Bud scale Number	Bud scale Per cent
Albania plantation	Co. 290	50	36	72.0	9	18.0	34	68.0
Billeaud plantation	Co. 290	100	6	6.0	0	0.0	11	11.0
Greenwood plantation	C.P.34/120	47	21	44.7	3	6.4	22	46.8
Little Texas plantation	C.P.36/183	45	3	6.7	0	0.0	1	2.2
Sterling plantation	Co. 290	100	64	64.0	32	32.0	67	67.0
		Cryolite Dusted Co. 290						
Sterling plantation		100	53	64.0	6	6.0	71	71.0

The red rot organism was isolated from nodes of the three varieties tested; however, it was isolated from only three leaf scars and one bud scale of C.P. 36/183. A difference was found in the number of isolations made from different plantations. With Co. 290 cane from Albania plantation, out of 50 notes plated, 36 leaf scars, 9 buds and 34 bud scales gave the organisms. The fungus was isolated from only 6 leaf scars and 11 bud scales when 100 nodes of Co. 290 cane from Billeaud plantation were plated. The number of isolates from C.P. 34/120 cane from Greenwood plantation was very high. Out of 47 nodes, the organism was cultured from 21 leaf scars, 3 buds and 22 bud scales.

Dusting with Cryolite did not affect the number of isolations made from Co. 290 cane from Sterling plantation. From 100 nodes, the leaf scars gave 53 cultures, from the buds, 6 cultures, and from the bud scales, 71 cultures. When 100 nodes of undusted Co. 290 cane from the same location were plated, the organism was isolated from 64 leaf scars, 32 buds and 67 bud scales.

Development of Red Rot in Cane Stalks of Different Varieties

This study was undertaken to determine the amount of latent infection of red rot present in different varieties of sugarcane. It had already been shown from previous tests that red rot developed in apparently healthy stalks of susceptible and resistant varieties when the cane was placed under suitable conditions. Stalks of different varieties used in this test were stored at 70°F. for approximately one month. After storage, the canes were examined for development of the organism from latent infections. At the same time, the initial points of entry of the organism into the stalk were studied. This was done by splitting the stalk longitudinally through the bud to study bud infection. Then the leaf scar tissue was removed with a knife to study leaf scar infection. Varieties used and results obtained are given in Table IV.

Table IV. Development of Red Rot in Stalks of Different Varieties of Sugarcane. Canes Placed in Storage at 70°F. for Approximately 30 days.

Variety	Nodes examined Number	Type of cane	Infection of node through Leaf scar Number	Leaf scar Per cent	Bud Number	Bud Per cent	Both leaf scar and bud Number	Both leaf scar and bud Per cent
D-74	100	Plant	30	30.0	16	16.0	0	0.0
Co. 290	165	Plant	38	23.0	21	12.7	7	4.2
Co. 290	200	Greenhouse	0	0.0	0	0.0	0	0.0
C.P. 34/120	200	Plant	22	11.0	19	9.5	2	1.0
C.P. 29/320	120	Plant	49	40.8	23	19.1	13	10.8
C.P. 36/19	100	Stubble	9	9.0	3	3.0	0	0.0
C.P. 26/116	110	Stubble	6	5.5	0	0.0	0	0.0
C.P. 36/105	125	Plant	2	1.6	0	0.0	0	0.0
C.P. 36/183	115	Stubble	3	2.6	0	0.0	0	0.0
C.P. 29/120	100	Plant	1	1.0	0	0.0	0	0.0
C.P. 36/13	100	Plant	0	0.0	0	0.0	0	0.0

From the above results it can be seen that cane varieties D-74, Co. 290 (plant cane), C.P. 29/320, and C.P. 34/120 had the greatest amount of latent infection. These four varieties are susceptible to red rot. Other varieties listed, as shown in the table, are considered resistant to red rot. This resistance is reflected in the amount of latent infection in the stalk. Out of 100 nodes of C.P. 36/13 (very resistant), none developed the disease.

Except for 3 growth ring infections which occurred in cane variety C.P. 29/320, the initial point of infection in all varieties could be traced to either the leaf scar or bud. For example, of 120 nodes of variety

C.P. 29/320, 19.1 per cent of the infections could be traced to the bud as the point of initial infection, 40.0 per cent to the leaf scars and 10.8 per cent to nodes having both bud and leaf scar infections. Out of 165 nodes of variety Co. 290, 39.9 per cent of the nodes developed red rot. Of these 12.7 per cent traced back to the bud, 23.0 per cent of the leaf scars and 4.2 per cent to nodes having both bud and leaf scar infections.

Upon examination of 165 nodes of variety Co. 290 plant cane grown in the field, it was found that red rot had developed in a higher percentage of the nodes than in greenhouse grown Co. 290 as determined by examination of 200 nodes. There was no infection in the cane grown in the greenhouse.

Effect of Different Fungicides on Development of Red Rot in Cane Stalks

Previous work showed that apparently healthy stalks were infected with the red rot organism in a dormant or latent form. To further check this conclusion, and to check the effect of different fungicides on development of the disease in the stalk, an experiment was made in which various fungicides were used to treat Co. 290 cane. After treatment, the fungicides were left on the stalks, which were then stored at 70°F. for approximately one month. The stalks were treated for 5 minutes in a one per cent suspension of either Tersan, Spergon or Phygon, and a 1-500 solution of Puratized N5E. Stalks of varieties Co. 290 and C.P. 34/120 were also treated for 30 minutes in a solution of bichloride of mercury before storage with the sterilizing agent left on the cane. The data are given in Table V.

Table V. Development of Red Rot in Stalks of Cane Varieties Co. 290 and C.P. 34/120 Subsequent to Treatment with Different Fungicides.

Treatment	Nodes examined	Infection of node through: Leaf scar Number	Bud Number	Growth ring Number	Both leaf scar and bud Number	Nodes infected Per cent
			Co. 290			
1 per cent Tersan 5 min.	104	27	19	4	0	48.0
1 per cent Spergon 5 min.	135	52	11	0	0	46.7
1 per cent Phygon 5 min.	100	33	8	3	2	46.0
1-1000 $HgCl_2$ for 30 min.	100	29	27	2	3	61.0
No treatment	103	26	16	2	0	44.0
1-500 Puratized N5E 5 min.	215	30	16	0	2	22.3
No treatment; check for Puratized treatment	212	59	33	0	10	48.1
			C.P. 34/120			
1-1000 $HgCl_2$ for 30 min.	110	8	6	1	2	15.4
No treatment	200	22	18	0	3	21.5

Red rot developed in both varieties regardless of treatment. In both treated and untreated stalks in which red rot developed, the initial points of infection could be traced to either the leaf scar or to the bud. This further confirmed the results obtained in previous tests. There was no significant reduction in the number of infected nodes between stalks of Co. 290 cane treated with either Tersan, Spergon, Phygon or bichloride of

mercury, and the untreated check. In fact, 61.0 per cent of the nodes treated with the mercury solution developed the disease, while the check cane had only 44.0 per cent of its nodes infected. The only Co. 290 cane treatment which gave a significant reduction was the 1-500 Puratized N5E treatment. Of 215 nodes treated, 22.3 per cent developed red rot, while out of 212 untreated nodes, 48.1 per cent became infected with the organism. This reduction was probably due to the fact that Puratized is a penetrating agent and it was able to destroy some of the dormant infections present in the leaf scars and bud scales.

Studies on Nature of Bud Infections and Bud Scale Penetration

By the isolation technique used in these studies, it has been shown that the red rot fungus can be cultured from the bud scales even after 24 hours immersion in bichloride of mercury. In order to obtain some information on how the bud scales became infected, the following was done. Young bud scales of cane varieties C.P. 36/13, Co. 290 and C.P. 34/120, grown in the greenhouse, were inoculated with a spore suspension of the fungus and then placed in a moist chamber for 33, 50, 60 and 77 hours, respectively. After incubation, the scales were removed from the buds and sectioned according to the procedure outlined in materials and methods. The sections were stained with cotton blue in lacto-phenol. Microscopic examination for appressoria and infection threads was made 3 or 4 hours after the sections were placed in the stain.

With the variety C.P. 34/120 (33 hours after inoculation), it was observed that appressoria had formed on the surface of the scale and from the contact surface of these structures, small pegs were found penetrating the wall of the epidermal cells. In cases in which the peg had entered

the inside of the cell a cap or overgrowth had formed around the peg. In areas in which the cell wall was thick, penetration was limited to the surface cells, although in areas without thick-walled cells the red rot fungus mycelium was observed invading the cells four or five cells below the epidermis.

Upon examination of inoculated bud scales of Co. 290 cane, it was observed that the same peg and overgrowth found in variety C.P. 34/120 were also present. The cap was also found in the epidermal cells of the bud scales of variety C.P. 36/13. With this resistant variety, the cap was found in the subepidermal cells. This was not observed with the varieties C.P. 34/120 and Co. 290.

In both Co. 290 (susceptible) and C.P. 36/13 (resistant) varieties, a dark gummy material was observed in the intercellular spaces in advance of the mycelium. It was noticed that this material was present in lesser amounts in Co. 290 cane.

In an attempt to obtain more information on how bud infection occurred, another experiment was undertaken. Stalks of Co. 290 cane were planted in the greenhouse and when the shoots were 3 or 4 inches tall they were dug and stored at 70°F. for two weeks. Upon examination of the shoots it was observed that initial infection occurred at the base of the shoots where they came in contact with the old bud scales. From this point the infection was found to spread towards the center of the shoot and down into the mother stalk. Further progress of the infection resulted in the death of the shoot and spread of the fungus throughout the cane stalk.

Results of Removing Tight and Green Leaf Sheaths from Growing Cane Stalks on Isolations of *Physalospora tucumanensis*

In this experiment, an attempt was made to determine whether or not latent infections in the stalks of varieties Co. 290 and C.P. 34/120 might be reduced by removing the leaf sheaths from growing cane before they became infected with the red rot fungus. To do this, the leaf sheaths were removed when they started to pull away from the stalk. This was done throughout the growing season. The leaf scars, buds and bud scales from these stalks were later plated on oatmeal agar after surface sterilization for 10 minutes in a solution of bichloride of mercury followed by calcium hypochlorite. The results are shown in Table VI.

Table VI. Effect of Removing Leaf Sheaths from Growing Cane While Green on Isolation of *P. tucumanensis* from the Leaf Scars, Buds and Bud Scales.

Treatment	Nodes plated Number	*P. tucumanensis* from Leaf scar Per cent	Bud Per cent	Bud scale Per cent
		Co. 290		
Leaf sheaths removed	150	.7	22.0[1]	.7
Leaf sheaths not "	150	69.3	42.0[1]	76.0
		C.P. 34/120		
Leaf sheaths removed	50	0.0	0.0	18.0
Leaf sheaths not "	50	24.0	6.0	22.0

[1] Only 50 buds plated.

Removal of the leaf sheaths before they became infected practically eliminated the latent infection in the leaf scars and bud scales. However, this was not true for the buds of Co. 290 cane. Out of 50 buds plated, 22.0

per cent gave the red rot fungus. The reason for this high rate of bud infection as compared to the leaf scar and bud scale infection was probably due to the fact that the buds protruded from the stalk after the leaf sheaths were removed. Fungus conidia could lodge between the bud and stalk and upon germination infect the young bud.

Of 150 nodes of Co. 290 cane in which the leaf sheaths were removed, only .7 of 1 per cent of the leaf scars gave the organism, while a corresponding number of nodes in which the sheaths were not removed gave 69.3 per cent infection. When bud scales from the same 150 nodes of Co. 290 cane in which the sheaths were removed were plated, the organism was isolated from .7 of 1 per cent of the scales. Of 150 bud scales from stalks in which the sheaths were not removed 76.0 per cent were found to be infected with the red rot fungus. With C.P. 34/120 cane it was found that the organism could not be isolated from the leaf scars and buds of nodes in which the leaf sheaths had been removed, but was isolated from 18.0 per cent of the bud scales. Those nodes from which the sheaths were not removed gave the organism from 24.0 per cent of the leaf scars, 6.0 per cent of the buds and 22.0 per cent of the bud scales. The data are shown in Table VI.

Cane stalks with the leaf sheaths removed and not removed were placed in storage at 70°F. for approximately 30 days to determine the extent to which red rot would develop in the stalk. This was done to find out whether or not there was any correlation between the number of isolates cultured and the number of bud and leaf scar infections developing in stalks placed in storage.

Results of this test are given in Table VII.

Table VII. Effect of Removing Leaf Sheaths from Growing Cane While Green on Development of P. tucumanensis in Stalks of Co. 290 and C.P. 34/120 Cane Held at 70°F. for One Month.

Treatment	Nodes examined Number	Infection of node through: Leaf scar Per cent	Bud Per cent	Nodes with both leaf scar and bud infection Per cent
		Co. 290		
Leaf sheaths removed	530	8.1	13.0	0.0
Leaf sheaths not "	350	60.3	41.3	10.6
		C.P. 34/120		
Leaf sheaths removed	150	6.7	6.7	0.0
Leaf sheaths not "	125	3.2	2.4	0.0

Stalks of Co. 290 cane which had their leaf sheaths removed developed red rot in 8.1 per cent of the leaf scars and 13.0 per cent of the buds, while those from which the sheaths were not removed had 60.3 per cent of the leaf scars and 41.3 per cent of the buds infected. When stalks of C.P. 34/120 cane were examined, it was found that both those with sheaths removed and sheaths not removed had developed red rot in only a few nodes. The results have shown that there was a correlation between the number of isolates cultured and development of red rot in the stalk. The data also showed that the number of latent infections in the stalk could be greatly reduced by removing the sheaths before they became infected.

Occurrence of Conidia and Perithecia of the Red Rot Fungus on Leaf Sheaths and Leaves of Sugarcane

This phase of the survey was undertaken to determine whether or not there was any correlation between susceptibility to red rot and the

occurrence of the conidial stage of P. tucumanensis on the leaf sheaths. Green sheaths of 9 varieties were collected and incubated in moist chambers for five days before examination. After incubation, the occurrence of the fungus spores on the insides of the sheaths was determined by microscopic examination. The sheaths were collected immediately after they had started to pull away from the stalk. The varieties used and results of the survey are given in Table VIII.

Table VIII. Occurrence of the Conidial Stage of the Red Rot Fungus on Green Leaf Sheaths of Different Varieties of Sugarcane.

Variety	Type of resistance	Leaf sheaths examined Number	Leaf sheaths found infected Number	Leaf sheaths infected Per cent
Co. 290	Susceptible	126	66	52.1
C.P. 34/120	Susceptible	30	11	36.7
C.P. 29/120	Resistant	30	12	40.0
C.P. 29/320	Intermediate	30	10	33.3
C.P. 36/105	Resistant	30	11	36.7
C.P. 36/19	Intermediate	30	16	53.3
C.P. 36/13	Resistant	20	6	30.3
C.P. 29/116	Resistant	20	7	35.0
C.P. 36/183	Resistant	21	9	42.9

The incidence of the fungus conidia on the sheaths was about the same for all varieties regardless of their resistance or susceptibility to the red rot fungus. Of 126 sheaths of Co. 290 cane examined, 66, or 52.1 per cent, were found infected. The next highest variety was C.P. 36/19 with 16 sheaths infected out of 30, or 53.3 per cent. For the resistant varieties examined, the conidia were found on an average of 35.0 per cent of the

sheaths examined. The results indicated that the number of infected leaf sheaths was not an indication of disease susceptibility.

Part of this experiment was devoted to an examination of dry or mature leaf sheaths of Co. 290 cane. This examination was made to determine the frequency of occurrence of the conidial and perithecial stage of the fungus under field conditions. Sheaths were collected from five locations and examined immediately after being collected. Results are summarized in Table IX.

Table IX. Occurrence of the Perithecial and Conidial Stages of *P. tucumanensis* on Mature Leaf Sheaths and Leaves of Co. 290 Cane Collected from Five Locations.

Location	Mature sheaths examined	Sheaths with perithecial and conidial stage		Leaves with perithecial and conidial stage	
	Number	Number	Per cent	Number	Per cent
L.S.U. Station	67	18	26.9	30	47.8
Albania plantation	35	34	97.1	26	74.2
Cecelia plantation	17	16	94.2	10	58.8
Ruth plantation	6	0	0.0	0	0.0
Total	125	68	-	66	-
Average	-	-	54.4	-	52.8

Both the conidial and perithecial stages were found on a high percentage of the leaf sheaths examined. Out of 125 leaf sheaths examined, 54.4 per cent were infected with both stages. About the same percentage of the leaves were found infected with both the conidial and the perithecial stages.

Results of Dusting Co. 290 Cane with Fermate, Zerlate and Parmate

The data from previous tests indicated that the red rot fungus was present in the leaf scars and bud scales in a dormant or latent form. The fact that the organism was absent in nodes having tight and clean sheaths (40) and also the fact that it could almost be eliminated from the stalk by stripping the leaf sheaths before infection had occurred led to tests which were undertaken in an attempt to reduce infection of the nodes by means of a dust applied behind the leaf sheaths during the growing season. With the idea of keeping a film of dust on the interior of the sheath throughout the growing season the dusts were applied every two weeks. This was done to prevent infection of the sheaths and bud scales by conidia which might be washed down from the leaf blade and midrib.

The fungicidal dusts Fermate (Ferric Dimethyldithiocarbamate), Zerlate (Zinc Dimethyldithiocarbamate), and Parmate (Zinc ethylene bisdithiocarbamate) were chosen because they had been reported as giving good results against other anthracnose fungi. A 10 per cent dust of each fungicide was prepared, using pyrax as the carrier. The first application was made with a hand duster after the leaf sheath surrounding the basal node had started to pull away from the stalk. Each time the cane was dusted caution was taken to make sure that the dust was applied behind sheaths surrounding the uppermost nodes of the stalk.

In order to determine whether or not the three different dusts were effective in reducing the amount of latent infection in the stalk, three separate tests were used. In the first test, leaf scars, buds and bud scales of dusted and non-dusted nodes were plated after 10 minutes in a bichloride of mercury solution followed by immersion in a solution of calcium hypochlorite. The number of isolates obtained from dusted nodes

was compared with the number isolated from the non-dusted nodes. By using this technique, it was possible to evaluate the effectiveness of the dust in reducing latent infection in the stalk. Results of isolations are given in Table X.

Table X. Isolation of Physalospora tucumanensis from Co. 290 Cane Dusted with a 10 Per Cent Dust of Fermate, Zerlate, or Parzate.

Treatment	Nodes plated	Nodes giving P. tucumanensis from					
		Leaf scar		Bud		Bud scale	
	Number	Number	Per cent	Number	Per cent	Number	Per cent
Fermate dusted	233	43	18.5	3	1.3	62	26.6
Zerlate dusted	210	41	19.5	3	1.4	34	16.2
Parzate dusted	200	40	20.0	6	3.0	38	19.0
Non-dusted	155	106	68.4	12	7.7	109	70.3

Results of plating the leaf scars, buds and bud scales gave a good indication of the effectiveness of the dusts. Out of 155 notes of undusted cane planted, 68.4 per cent of the leaf scars, 7.7 per cent of the buds and 70.3 per cent of the bud scales gave the organism. When stalks receiving the dust were plated, a reduction in number of infected nodes was noted. For example, the organism was isolated from only 20.0 per cent of the leaf scars, 3.0 per cent of the buds, and 19.0 per cent of the bud scales when Parzate was used. The other two dusts were found to be as effective as Parzate in reducing latent infection in the stalk.

The second test consisted of placing dusted and non-dusted stalks in storage at 70°F. for approximately 30 days. After this period of storage, the buds and leaf scars were removed and the number of infected nodes recorded. By this method, it was possible to study the effect of the three

dusts on the development of red rot in the stalk. Results are given in Table XI.

Table XI. Development of Red Rot in Dusted and Non-dusted Stalks of Co. 290 Cane Held at 70°F. for Approximately 30 Days.

Treatment	Nodes examined	Infection of node through: Leaf scar Number	Leaf scar Per cent	Bud Number	Bud Per cent	Total nodes Number	Total nodes Per cent
Zerlate dusted	130	9	6.9	8	6.2	17	13.1
Fermate dusted	106	9	8.5	15	14.1	24	22.6
Parzate dusted	108	8	7.4	12	11.1	20	18.5
Undusted	273	68	24.9	42	15.4	110	40.3

In 273 nodes of non-dusted cane, 40.3 per cent of the nodes showed infection by the organism. Nodes receiving the dusts showed a reduction in red rot. In the case of those dusted with Zerlate, only 13.1 per cent developed the disease. The Fermate dusted cane showed 22.6 per cent infection and the Parzate dusted cane showed 18.5 per cent infection.

The purpose of the third test was to determine whether or not stands of Co. 290 cane might be improved by the use of dusted seed cane; the two previous tests having shown that there was a reduction in the amount of latent infection in the dusted stalks. Therefore, the use of these stalks for planting purposes might help stabilize the stand in the spring.

Plantings were made at two different dates. The first planting was made October 15 and the second November 11. Non-infected Co. 290 cane grown in the greenhouse was also included in the test. Plots 25 ft. long were used and each treatment was replicated four times. One hundred twenty apparently healthy eyes were planted per plot. The stand count and yield of the two tests are given in Tables XII and XIII.

Table XXI. Effect of Dusting Co. 290 Cane with Zerlate, Fermate, and Parzate on Stand Count in the Spring.

Replication	Check	Greenhouse	Parzate	Fermate	Zerlate
		Stand Count Test I			
1	17	40	32	19	9
2	21	33	30	24	40
3	27	31	36	25	20
4	11	43	32	21	24
Total	76	147	130	89	93
Average	19.0	36.8	32.5	22.3	23.3
Difference		+94%	+71%	+17%	+23%
		Stand Count Test II			
1	15	28	2	8	13
2	18	2	14	34	30
3	22	30	33	20	13
4	34	29	27	19	4
Total	89	89	76	81	60
Average	22.3	22.3	19.0	20.3	15.0
Difference		0.0	-15%	- 9%	-33%

The greenhouse Parzate, Fermate, and Zerlate treated plantings in the first test gave increases over the non-dusted check. Cane from the greenhouse gave a 94 per cent increase in stand, while Parzate was second with 71.0 per cent increase. Fermate and Zerlate gave increases in stand of 22.3 and 23.3 per cent, respectively.

The second test was planted in November in a low spot in which drainage was poor. Due to this late planting, the cane did not germinate until the spring of the next year. In the spring when all the cane had emerged, it was observed that in some of the replications the stand was very poor. Some of the cane was dug and examined in an attempt to determine the cause of the trouble. The interior of the cane appeared watersoaked and had a salmon color. Upon plating, fungi belonging to the genus Phytophthora were isolated from the interior of the stalks. The red rot fungus was not isolated from this cane. Inoculation of this fungus into healthy seed pieces resulted in a similar reduction of stand. Since P. tucumanensis was not isolated from the plated cane, it was concluded that the poor stand in test 2 was due to the deterioration of the seed pieces by the Phytophthora.

As in the stand count, the greenhouse Parzate, Fermate, and Zerlate treated plantings in the first test gave increases over the non-dusted check. In this case, Zerlate gave a 16.0 per cent increase in tonnage, while Parzate was next with a 14.0 per cent increase. Yield of the Fermate plots was 10.0 per cent greater than the check. The greenhouse cane was lowest, with a 9.0 per cent increase.

Yield data from test 2 were not considered valid because it was shown that Phytophthora caused a deterioration of the seed pieces.

Table XIII. Effect of Dusting Seed Cane with Zerlate, Fermate, and Parzate on Yield of Co. 290 Cane.

Replications	Check	Greenhouse Parzate	Fermate	Zerlate
		Yields in tons: Test I		
1	23.2	26.1	28.3	34.1	32.7
2	25.4	21.7	23.2	31.2	37.7
3	28.3	27.5	36.2	29.7	23.2
4	29.0	39.9	35.5	21.8	29.0
Total	105.9	115.2	123.2	116.8	122.6
Average	26.5	28.8	30.1	29.2	30.7
Difference		+ 9%	+14%	+10%	+16%
		Yields in tons: Test II		
1	13.0	18.2	17.8	20.3	23.2
2	12.3	23.2	14.5	22.5	20.3
3	22.5	18.4	15.2	26.1	12.3
4	26.1	22.5	22.8	23.2	11.9
Total	73.9	82.9	70.3	92.1	67.7
Average	18.5	20.7	17.6	23.0	16.9
Difference		+12%	- 5%	+24%	- 9%

Results of Dusting and a Seed Treatment on Stand and Yield of Co. 290 Cane

In an attempt to obtain some information regarding the use of a dust and seed treatment on cane to be planted, the following was done. Before planting, canes dusted with either Fermate, Zerlate, or Parzate were dipped in a 1 per cent Tersan solution for 5 minutes. A like amount of

cane which received only the dust was planted for comparison. Cane without dust or seed treatment was used for the check. Each treatment was replicated four times, using 120 healthy eyes per 25 ft. section. Results of stand count and yield are given in Table XIV.

Table XIV. Effect of Dusting and a Seed Treatment of Tersan on Stand and Yield of Co. 290 Cane.

Replication	Check	Parmate plus Tersan	Parmate only	Fermate plus Tersan	Fermate only	Zerlate plus Tersan	Zerlate only
				Stand count			
1	8	35	13	4	7	3	3
2	18	0	9	21	5	5	3
3	1	46	20	7	19	11	6
4	2	23	28	13	4	10	10
Total	29	104	70	45	35	29	22
Average	7.3	26.0	17.5	11.2	8.8	7.3	5.5
Difference		+256%	+140%	+53%	+20%	0	+25%
				Yield in Tons			
1	5.0	21.7	22.0	19.1	5.5	18.1	8.0
2	8.1	21.7	25.4	28.1	23.6	15.2	11.2
3	23.5	10.1	10.0	27.8	11.6	19.6	10.2
4	4.2	29.0	22.4	3.5	13.2	4.9	4.1
Total	40.8	82.5	79.8	78.5	53.9	57.8	33.5
Average	10.2	20.6	19.9	19.6	13.7	14.4	8.6
Difference		+102%	+95%	+92%	+34%	+41%	-16%

Dusted canes receiving the Tersan seed treatment gave increases over dusted and non-dusted check. The treatment, Parzate plus Tersan, gave 256.0 per cent increase in stand and 102.0 per cent increase in tonnage over the undusted check. Cane receiving Parzate only showed a 140.0 per cent increase in stand and a 95.0 per cent increase in yield. All other treatments, except Zerlate without seed treatment, showed increases in stand and yield. Increases obtained with Parzate plus Tersan, Parzate only and Fermate plus Tersan were highly significant according to a statistical analysis of the data.

Results of Dusting Co. 290 Cane with Parzate at Four Locations

The use of a dust during the growing season to control red rot in Co. 290 cane showed promise in preliminary trials. By the plating technique, it was shown that latent infection in the nodal region could be reduced by two-thirds by dusting. Development of bud and leaf scar infections in dusted cane placed in storage was also reduced. Results of planting tests indicated that increases in stand and yield might be obtained by dusting.

To follow this further, dusting tests were conducted at Louisiana State University, Youngsville, Billeaud, and Martin Ridge plantations, using a 10 per cent Parzate dust. Frequency and method of application used were the same as outlined in the previous dusting experiment. Dusting was started on July 1 and continued throughout the growing season. At the end of the growing season, about planting time, cane was collected from the different locations and tested to determine whether or not there was a reduction in the amount of latent infection in the stalk. Testing was done by the methods used in the previous test.

Results are summarized in Table XV and XVI.

Table XV. Occurrence of the Red Rot Fungus in Leaf Scars, Buds, and Bud Scales of Co. 290 Cane from Four Locations. Cane Dusted Every Two Weeks with a 10 Per Cent Parzate Dust.

Treatment	Location	Nodes plated Number	Nodes giving P. tucumanensis from Leaf scar Number	Leaf scar Per cent	Bud Number	Bud Per cent	Bud scale Number	Bud scale Per cent
Dusted	Billeaud plantation	100	3	3.0	0	0.0	17	17.0
Undusted	Billeaud plantation	100	37	37.0	1	1.0	52	52.0
Dusted	Martin Ridge plantation	150	86	57.4	15	10.0	111	74.0
Undusted	Martin Ridge plantation	100	58	58.0	7	7.0	84	84.0
Dusted	Youngsville plantation	100	24	24.0	-	-	52	52.0
Undusted	Youngsville plantation	100	38	38.0	-	-	70	70.0
Dusted	L.S.U. plots	150	24	16.7	-	-	44	29.3
Undusted	L.S.U. plots	150	75	50.0	-	-	100	66.7

Martin Ridge plantation was the only location in which there was not a reduction in the number of isolates obtained from the leaf scars, bud, and bud scales. Conditions at that location were ideal for development and spread of the fungus throughout the growing season. Frequent rains plus a heavy stand of cane favored the spread of fungus spores. When it was realized that dusting every two weeks was ineffective, it was increased to once a week. A later test showed that dusting once a week was better than once every two weeks.

Non-dusted cane from Billeaud plantation gave the organism from 37.0 per cent of the leaf scars, 1.0 per cent of the buds and 52.0 per cent of the bud scales. The dusted canes from the same plantation were much lower in infection, with 3.0 per cent from the leaf scars, none from the buds and

17.0 per cent from the bud scales. Tests at Louisiana State University and Youngsville showed a similar reduction in the amount of latent infection present in the nodal region of dusted stalks.

Table XVI. Development of Red Rot in Dusted and Non-dusted Stalks of Co. 290 Cane from Four Locations. Cane Dusted Every Two Weeks with a 10 Per Cent Parzate Dust.

Treatment	Location	Nodes examined	Infection of node through					
			Leaf scar		Bud		Total node	
		Number	Number	Per cent	Number	Per cent	Number	Per cent
Dusted	Billeaud plantation	200	74	37.0	32	16.0	106	53.0
Undusted	Billeaud plantation	200	92	46.0	65	32.5	157	78.5
Dusted	Martin Ridge plantation	200	116	58.0	40	20.0	156	78.0
Dusted	Martin Ridge[1] plantation	100	42	21.0	12	6.0	54	54.0
Undusted	Martin Ridge plantation	200	100	50.0	65	32.5	165	82.5
Dusted	Youngsville plantation	200	40	20.0	24	12.0	64	32.0
Undusted	Youngsville plantation	200	39	19.5	33	16.5	72	36.0
Dusted	L.S.U. plots	200	15	7.5	10	5.0	25	12.5
Undusted	L.S.U. plots	200	53	26.5	37	18.5	90	45.0

[1] Dusting was increased to once a week instead of every two weeks.

The highest reduction in number of nodes developing red rot resulted from the dusting made at Louisiana State University. Out of 200 nodes of dusted cane examined, 12.5 per cent showed infection at the node. A corresponding number of non-dusted nodes developed red rot in 26.5 per cent of the leaf scars and 18.5 per cent of the buds, or a total of 45.0 per cent of the nodes were infected. This decrease in number of infected nodes at the above location might have been due to the fact that the row was dusted on both sides instead of on one side as was the practice in the other

three plots. A greater amount of dust was also used. Cane at Martin Ridge plantation which was dusted every two weeks developed the disease in as many nodes as the non-dusted cane. However, when dusting was increased to once a week, there was a 28.5 per cent reduction. The dusted cane developed the disease in 54.0 per cent of the nodes tested, while the non-dusted cane showed 82.5 per cent infection. The cane lot from Youngsville failed to show a significant reduction.

Cane dusted at the four different locations was used for seed purposes at planting time. Planting tests were conducted at each location where cane was dusted. In addition, cane from Louisiana State University was used for a test at Albania plantation. In the tests at Albania, Youngsville and Billeaud, the dusted and non-dusted canes were planted using the alternate row method. The replicated plot technique was used for the Louisiana State University test. The test at Louisiana State University included three dates of planting. The first planting was made September 10, the second on October 13 and the last planting on December 15. The test at Martin Ridge plantation was conducted in cooperation with Mr. A. L. Dugas, Entomologist of the Louisiana Agricultural Experiment Station. In that test, Co. 290 cane was dusted, using Parzate alone, Parzate in combination with Cryolite, Cryolite alone and Chlorodane. Cryolite (Sodium fluoaluminate) and Chlorodane (Chlorinated hydrocarbon) are insecticides used against the sugarcane borer. This test will be discussed separately from the other four tests. Yield data for all planting tests will not be included in this manuscript because at the time of writing the yield data were not available. Shoot counts were made in the spring at four locations. The data are given in Table XVII.

Table XVII. Effect of Dusting Seed Cane of Co. 290 Cane with a 10 Per Cent Perzate Dust on Stand Count in the Spring. Count for 60 Feet of Row.

Row	Non-dusted Stand Count 1	2	Row	Dusted Stand Count 1	2
			Albania Plantation		
3	112	185	4	203	245
5	176	179	6	146	226
7	125	126	8	127	254
9	117	145	10	150	-
11	164	-	12	-	-
Total	1329			1351	
Average	265.8			337.8	
Per cent increase	27.0				
			Billeaud Plantation		
26	203	176	25	258	268
28	186	237	27	281	326
30	181	225	29	256	328
32	181	245	31	238	295
34	246	216	33	301	260
36	151	220	35	255	290
Total	1319			1767	
Average	411.1			559.3	
Per cent increase	36.0				

(continued)

Table XVII. (Continued)

Row	Non-dusted Stand Count	Row	Dusted Stand Count
		Youngsville Plantation	
5	125	4	148
7	160	6	106
9	116	8	129
11	135	10	147
13	155	12	121
15	95	14	132
17	132	16	131
19	100	18	81
21	92	20	115
23	81	22	100
Total	1191		1207
Average	119.1		120.7
Per cent increase	1.3		

L. S. U. Station

Replication	Non-dusted	Greenhouse	Farmers
	Stand Count September Planting		
1	200	240	169
2	170	105	150
3	100	160	110
Total	470	505	429
Average	156.6	168.3	143.0
Difference		+ 7.5%	- 8.7%

(continued)

Table XVII. (Continued)

Replication	Non-dusted	Greenhouse	Parsate
	Stand Count October Planting		
1	26	86	43
2	51	79	60
3	25	62	46
Total	102	227	149
Average	34.0	75.7	49.7
Difference		+122.6%	+ 46.2%
	Stand Count December Planting		
1	55	-	53
2	53	-	51
3	56	-	63
Total	164	-	167
Average	54.7		55.7
Difference			+ 0.2%

Planting tests on two of the three plantations showed significant increases in shoot counts made in the spring. At Billeaud plantation there was a 36.0 per cent increase in stand. The shoots on the dusted rows also appeared more vigorous than the adjoining shoots of the non-dusted rows. Cane from Louisiana State University planted at Albania plantation gave a 27.0 per cent increase in the number of shoots present in the spring. The Youngsville test, however, failed to show any increase. In that test it was noted that the cane was planted deeper than is customary (6 to 10 inches) and none of the shoots emerged in the fall. In the spring, the

cane was very late in germinating. This was apparently due to the depth at which the cane was planted. Interior portions of stalks from which no shoots had emerged were plated and it was found that Phytophthora was present in many of the internodes tested. The failure of this test to give an increase in shoot count might have been due both to the depth of planting and to Phytophthora.

The September and December plantings at Louisiana State University did not give a significant increase in stand. In the September test, the greenhouse cane was only 7.5 per cent better than the non-dusted check. The Parzate dusted cane in the same test showed a 8.7 per cent reduction. The Parzate cane in the December test was only 0.2 per cent better than the non-dusted check. Cane planted in these two tests was not examined for the presence of Phytophthora, yet when cane from adjoining rows which had failed to germinate were plated, the organism was cultured from many of the internodes. The October tests was the only one in which there was a significant increase in shoot count. A count of the shoots from the greenhouse cane showed that 122.6 per cent more shoots had emerged from the greenhouse cane than from the non-dusted cane. Parzate cane also gave a 46.7 per cent increase over the non-dusted cane.

Results of Dusting Co. 290 Cane with Parzate, Cryolite and Chlorodane and a Combination of Parzate and Cryolite

Cryolite, which is used to control the sugarcane borer (Diatraea saccharalis), is used extensively in areas where Co. 290 cane is grown in Louisiana. In an attempt to obtain some information on the effect of Cryolite, Chlorodane, Parzate and Parzate in combination with Cryolite, on latent infection of red rot in stalks of Co. 290 cane, a test was conducted

in which Co. 290 cane was dusted every two weeks with Parzate and weekly for four weeks with Cryolite and Chlorodane. The Cryolite and Chlorodane dusts were applied weekly for four weeks whenever necessary. Parzate was applied every two weeks until it was found necessary to increase the dusting to once a week. At the end of the growing season, about planting time, canes from the various treatments were brought to the laboratory and tested, using the techniques described in the previous experiments. A planting test, using the replicated plot technique, was conducted to determine whether or not any of the treatments would improve the stand of Co. 290 cane in the spring. Stand counts of this test were made late in the spring. Results of the different tests are given in the following tables.

Table XVIII. Occurrence of the Red Rot Fungus in the Leaf Scars, Buds and Bud Scales of Co. 290 Cane from Martin Ridge Plantation. Cane Dusted with a 10 Per Cent Parzate Alone and in Combination with Cryolite.

Treatment	Nodes plated	Nodes giving the fungus from					
		Leaf scar		Bud		Bud scale	
	Number	Number	Per cent	Number	Per cent	Number	Per cent
Parzate only	100	65	65.0	8	8.0	80	80.0
Parzate only[1]	50	21	42.0	-	-	31	62.0
Parzate plus Cryolite	100	71	71.0	7	7.0	74	74.0
Cryolite only	150	58	38.7	7	4.7	88	58.7
Non-dusted	100	58	58.0	7	7.0	84	84.0

[1] Dusting increased to once a week instead of every two weeks.

The treatment, Parzate only, did not cause a significant reduction in the number of infected nodes when the dust was applied every two weeks. However, when the dust was applied once a week there was a 16.0 per cent reduction

in the number of infected leaf scars and 22.0 per cent in the number of infected bud scales. The Cryolite treatment showed the greatest reduction of infected nodes. Of 150 nodes plated, the organism was cultured from 38.7 per cent of the leaf scars, 4.7 per cent of the buds and 58.7 per cent of the bud scales. When the non-dusted cane was plated, the organism was isolated from 58.0 per cent of the leaf scars, 7.0 per cent of the buds and 84.0 per cent of the bud scales.

Table XIX. Development of Red Rot in Stalks of Co. 290 Cane from Martin Ridge Plantation. Cane Dusted with Chlorodane, 10 Per Cent Parzate Alone and a Combination of Parzate and Cryolite. Cane Held at 70°F. for Approximately 30 Days.

Treatment	Nodes examined	Infection of nodes through				Total nodes infested	
		Leaf scars		Buds			
	Number	Number	Per cent	Number	Per cent	Number	Per cent
Chlorodane	200	80	40.0	65	32.5	145	72.5
Cryolite only	200	70	35.0	38	19.0	108	54.0
Parzate only	200	116	58.0	40	20.0	156	78.0
Parzate only	100[1]	42	42.0	12	12.0	54	54.0
Parzate plus Cryolite	200	92	46.0	37	18.5	129	64.5
Non-dusted	200	100	50.0	65	32.5	165	82.5

[1] Dusting increased to once a week instead of every two weeks.

Stalks of Co. 290 cane which were dusted weekly and those receiving Cryolite only gave the greatest reduction in the number of nodes developing red rot. The Cryolite plus Parzate dusted cane also showed a reduction in number of leaf scars and buds developing the disease. Of 200 nodes examined, 64.5 per cent of the nodes showed infection by the organism. The same number of non-dusted nodes showed infection by the red rot fungus in 50.0 per

cent of the leaf scars, and 32.5 per cent of the buds, or a total of 82.5 per cent of the nodes developed the disease.

Table XX. Results of Analyses of Variance of Planting Test with Co. 290 Cane Dusted with Chlorodane, 10 Per Cent Parzate Alone, and a Combination of Parzate and Cryolite. Two Soil Types and Two Dates of Planting Used.

Sources of Variation	Degrees of freedom	Sum of squares	Mean square	F
Soil Types	1	40,049	40,049.00	8.24**
Dates of Planting	1	333,851	333,851.00	68.73**
Soil x Dates	1	232,850	232,850.00	47.94**
Treatments	4	65,313	16,328.25	3.36*
Treatment x Soil	4	18,450	4,612.50	
Treatment x Dates	4	15,365	3,841.25	
Treatments x Soil x Dates	4	2,917	729.25	
Replications	12	462,763	38,563.58	7.94**
Error	48	233,160	4,857.50	
Total	79	1,404,718		

* Denotes significance according to the F test.
** Denotes highly significant according to the F test.

Comparison of Treatments at Individual Locations

Light Soil Planting

Treatments	Early Planting Average Shoot Count	Late Planting Average Shoot Count
Non-dusted check	605.5	382.5
Cryolite only	710.3*	425.5**
Cryolite plus Parzate	656.3*	385.0
Parzate only	578.3	394.5
Chlorodane	638.5	415.8

* Difference of 49.62 required for significance at the 5% level.
** Approaches significance.

Comparison of Treatments at Individual Locations (continued)

Heavy Soil Planting

Treatments	Early Planting Average Shoot Count	Late Planting Average Shoot Count
Non-dusted check	521.0	506.8
Cryolite only	652.0*	582.5*
Cryolite plus Parzate	566.8**	554.2**
Parzate	593.3*	573.2*
Chlorodane	539.2	549.8

* Difference of 49.62 required for significance at the 5% level.
** Approaches significance.

Comparison of Dates of Planting

Light Soil

Treatments	Early Planting Average Shoot Count	Late Planting Average Shoot Count
Non-dusted check	605.5*	382.5
Cryolite only	710.3*	425.5
Cryolite plus Parzate	656.3*	385.0
Parzate	578.3*	394.5
Chlorodane	638.5*	415.8

Heavy Soil

Treatments	Early Planting Average Shoot Count	Late Planting Average Shoot Count
Non-dusted check	521.0	506.8
Cryolite only	652.0*	582.5
Cryolite plus Parzate	566.8	554.2
Parzate	593.3	573.2
Chlorodane	539.2	549.8

* Difference of 31.37 required for significance at the 5% level.

Comparison of Soils and Dates of Planting

Treatments	Light Soil Early Planting Average Shoot Count	Heavy Soil Late Planting Average Shoot Count
Non-dusted check	605.5*	506.8
Cryolite only	710.3*	582.5
Cryolite plus Parzate	656.3*	554.2
Parzate only	578.3	573.2
Chlorodane	638.5*	549.8

Treatments	Light Soil Late Planting Average Shoot Count	Heavy Soil Early Planting Average Shoot Count
Non-dusted check	382.5	521.0*
Cryolite only	425.5	652.0*
Cryolite plus Parzate	385.0	566.8*
Parzate only	394.5	593.3*
Chlorodane	415.8	539.2*

Treatments	Light Soil Early Planting Average Shoot Count	Heavy Soil Early Planting Average Shoot Count
Non-dusted check	605.5*	521.0
Cryolite only	710.3**	652.0
Cryolite plus Parzate	656.3*	566.8
Parzate only	578.3	593.3
Chlorodane	638.5*	539.2

Comparison of Soils and Dates of Planting
(continued)

Treatments	Light Soil Late Planting Average Shoot Count	Heavy Soil Late Planting Average Shoot Count
Non-dusted check	382.5	506.8*
Cryolite only	425.5	582.5*
Cryolite plus Parzate	385.0	554.2*
Parzate	394.5	573.2*
Chlorodane	415.8	549.8*

* Difference of 62.76 required for significance at the 5% level.
** Approaches significance.

Comparison of Soils

Treatments	Light Soil Average Shoot Count**	Heavy Soil Average Shoot Count**
Non-dusted check	494.0	513.9
Cryolite only	567.9	617.3*
Cryolite plus Parzate	520.7	560.5*
Parzate	486.4	583.3*
Chlorodane	527.2	544.5

* Difference of 31.37 required for significance at the 5% level.
** Average for both early and late dates of planting.

An analysis of the data obtained from the planting test at Martin Ridge plantation showed that there were significant differences between soil types, and highly significant differences between dates of planting, soil and dates and treatments. A comparison of the treatments at individual locations, using the t test, showed that in the light soil early planting test, there were significant differences between the check and Cryolite only treatment and the check and Cryolite plus Parzate treatment. The

treatment, Cryolite only, in the light soil late planting test approached significance. Comparison of treatments in the heavy soil early planting test showed that there were significant increases with the treatments Cryolite only and Parzate only. The Cryolite plus Parzate treatment in the same test approached significance. The heavy soil late planting test gave the same results as the heavy soil early planting test.

In the comparison of dates of planting it was found that all the treatments in the early planting on light soil, including the non-dusted check, were significantly higher than those of the late planting test on the same soil. When the same comparison was made for heavy soil, the treatment, Cryolite only, in the early date of planting test was significantly higher than Cryolite only in the late date of planting test. Other date of planting comparisons (treatments) failed to show significant differences.

A comparison of soils and dates of planting showed that all treatments except Parzate in the light soil early planting test were significantly higher than the same treatments planted in the heavy soil late planting test. All treatments, including the non-dusted check, in the heavy soil early planting test were significantly higher than the same treatments in the light soil late planting test. A comparison of the same dates of planting tests but on different soil types showed that the treatments, Cryolite plus Parzate and Chlorodane, of the light soil early planting test gave significant increases over the same treatments in the heavy soil early planting test. The non-dusted check in the light soil early planting test was also significantly higher than the non-dusted check in the heavy soil early planting test. All treatments in the heavy soil late planting test were significantly higher than the treatments in the light soil late planting test.

The table shows that the treatments, Cryolite only, Cryolite plus Parzate and Parzate only, on heavy soil gave significant increases in shoot count over the light soil test in which the same treatments were used.

The Effect of Environment on Development of Red Rot in Stalks of Co. 290 Cane

Studies on effect of environment on development of red rot in sugar-cane stalks can be made because it has been shown that when the organism was present in the stalk, it remained in a latent form until conditions favored further invasion. In an attempt to determine whether or not different environmental conditions might affect the progress of the disease in the stalk, Co. 290 cane was placed under the different conditions listed in Table XXI. The stalks used were obtained from an area in which the red rot organism was known to be present. Previously the organism had been isolated from other stalks from the same area. After the stalks were kept under the various conditions listed in the table, the number of nodes developing either leaf scar or bud infection was determined. The number of infections of each type was used to evaluate the effect of environment on development of the disease in the stalk. The data are shown in Table XXI.

Table XXI. Development of Red Rot in Stalks of Co. 290 Cane Placed Under Different Environmental Conditions

Treatment	Nodes examined Number	Infection of node through Leaf Scar Number	Bud Number	Total nodes infected Per cent	Healthy buds Number
Cane kept watered in soil for 1 mo. at 80-90°F.	100	6	0	6.0	100
Cane kept watered in soil for 1 mo. at 70°F.	100	0	1	1.0	98
Cane kept outside in Dec. and Jan. in sterilized soil	90	1	4	5.6	86
Cane kept at 16-18°C. for 7 wks. and 1 wk. at room temperature	100	19	12	31.0	88
Cane kept in air-dried soil for 1 mo. at 80-90°F.	100 Leaf scars 22 buds	26	8	34.0	78
Cane kept in air-dried soil for 1 mo. at 70°F.	100	14	6	20.0	90
Cane kept outside in air-dried soil in Dec. and Jan.	100	16	11	27.0	89
Cane kept on floor of cold room at 70°F. for 1 mo.	100	17	11	28.0	0
Cane planted in soil (sterile) then covered with water for 2 wks. at 80-90°F.	100	1	2	3.0	19
Cane planted in soil (sterile) then covered with water for 2 wks., kept outside in Dec. and Jan.	100	2	0	2.0	90

Results of this experiment indicated that dry conditions favored the development of red rot in stalks of Co. 290. Of 100 nodes kept in air-dried soil for one month at 80-90°F., 26 of the leaf scars showed infection by the red rot fungus. Only 22 buds from the above 100 nodes were examined

and of these, 8 were infected. There was enough water in the air-dried soil to cause the remaining 78 buds to germinate. When the shoots developing from these buds were 3 or 4 inches in height, the mother stalks were dug and then placed in storage for two weeks at 70°F. with the shoots adhering to the stalks. Upon examination, it was found that of 66 shoots, 34 had developed red rot near the base of the shoot where they came in contact with the old bud scales. Stalks used in other treatments in which dry conditions prevailed, developed red rot, regardless of temperature. The four treatments in which moist conditions prevailed, apparently prevented development of the disease because the organism was found spreading in only a few nodes.

DISCUSSION

The manner in which the red rot fungus enters the sugarcane stalk has been studied by numerous investigators and the entry of the fungus into the stalk through the uninjured epidermis has been reported by several workers. Abbott (2) found that under certain conditions the fungus was capable of entering the stalk through the root band region. Padwick (34) and Howard (29) thought that the leaf scar could serve as a point of entry. The present studies failed to confirm the work of Abbott, but agreed with the conclusion of Padwick and Howard. That both the leaf scar and bud could serve as points of entry was reported earlier by Steib (40). Studies included in this manuscript further support this conclusion.

Previous studies by Steib (40) have shown that the organism is not merely on the surface of the cane, but is either in the waxy cuticle or in the tissue where a sterilizing agent cannot reach it. Isolation tests showed that the organism could be isolated from the root band and bud after as much as 24 hours immersion in bichloride of mercury. In these studies, it was shown that the fungus could also be isolated from the leaf scar and bud scales after the same period of sterilization. Development of the disease in stalks placed under unfavorable conditions after they were surface-sterilized, was again demonstrated in these studies. Steib (40) used this method and the isolation technique as evidence to support his conclusions that the red rot organism was present in the sugarcane stalk in a latent form. Other workers (6, 7, 8, 26, 36, 38, 42) have concluded that other diseases caused by either Colletotrichum or Gloeosporium may

occur in a latent form.

Isolation of the organism from the leaf scar, bud and bud scales of apparently healthy stalks after surface-sterilization suggested that infection of these tissues had occurred during the growing season. Steib (40) found that spores of the fungus were present on leaf sheaths which were loose around the stalk and also where they were tight around the stalk but red in color. However, none were found on sheaths near the top of the stalk that were tight and clean. Isolations showed that the organism could be cultured from nodes having infected sheaths and not from nodes having tight clean sheaths. These results indicated that the leaf scar, bud and bud scales became infected after the leaf sheaths became infected. During these studies an experiment was conducted in which the leaf sheaths were removed from the stalk before they became infected with the fungus. Plating of the leaf scars, buds and bud scales of nodes in which the sheaths had been removed showed that the organism was present only in a few nodes. The organism was isolated from a higher percentage of the buds than of the leaf scar and bud scales. This was probably due to the fact that the buds protruded from the stalk after the sheaths were removed. The spores of the fungus might then become lodged between the bud and stalk and upon germination infect the bud. The results of this experiment indicated that the fungus grew from the infected leaf sheaths into the leaf scar tissue. Previously Steib (40) reported that infection of the nodal region might occur through the vascular region of the leaf sheaths directly into the stalk. This was demonstrated by tracing red vascular tissue from the leaf scar into the stalk of cane held in storage. The organism was demonstrated by plating to have been present in these reddened streaks.

Isolation of the organism from the bud and bud scales after surface-sterilization and the development of bud-type infections in surface-sterilized stalks placed in storage, indicated that infection of these tissues had occurred sometime during the growing season. Results obtained from bud infection studies have shown that appressoria form on the surface of young bud scales and from the contact surface of the appressorium, a small thread was found penetrating the wall of the epidermal cell. This occurred 33 hours after the bud scales were inoculated with a conidial suspension of the fungus. In cases in which the thread had entered the inside of the cell, a cap or overgrowth formed around the peg. The cap was found to be present in the bud scales in two susceptible varieties and one resistant variety studied. However, with the resistant variety, the cap was found in both the epidermal and subepidermal cells. This was not observed with the other two varieties. Tests were not undertaken to determine the nature of the overgrowth; however, it appeared to be a product of the host cell wall. The cap was found to increase in size as the peg penetrated deeper into the cell and the peg was always found enclosed within the overgrowth. The presence of an overgrowth around the infection thread, the failure of the fungus to invade areas of thick-walled cells and the presence of the cap in subepidermal cells of a resistant variety might be considered as evidence of morphological resistance. In both the resistant and one of the susceptible varieties studied, a dark gummy material was produced in the intercellular spaces in advance of the mycelium. This material was produced in lesser amounts in the susceptible variety. Invasion of the cells beyond the area in which this material was present was not observed. This type of resistance might be considered physiological.

In the study on how bud infection occurs, it was observed that the red rot fungus remained dormant when the stalks were kept watered and in a

vigorous condition. Young shoots emerging from these stalks did not show symptoms of the disease until the mother stalks were placed in storage under dry conditions, with the shoots adhering to the stalk. Infection of the shoots occurred at the base where they came in contact with the old bud scales.

Experiments conducted in these studies have demonstrated that the fungus may gain entrance into the stalk through the leaf scar and bud. This was done by placing apparently-healthy stalks of a susceptible variety in storage for one month. Upon examination, it was found that red rot areas developed in the nodal regions and adjoining internodes and could be traced back to initial points of infection, the bud and leaf scar. The red rot organism was shown to be present by plating infected tissue.

It was also found during this research that surface-sterilization of the cane stalks with a solution of bichloride of mercury would not prevent development of the disease in the stalk. In both treated and non-treated stalks the organism was observed spreading from the initial points of entry, the bud and leaf scar, into the internodal region of the stalk. These results further support the results reported earlier by Steib (40).

Table V shows that treatment of the stalk with Tersan, Spergon, Phygon, 1-1000 solution of bichloride of mercury and Puratized N5E did not prevent development of the fungus in the stalk. However, the Puratized N5E treatment gave somewhat lower percentages of infected nodes with variety Co. 290. This reduction was probably due to the fact that Puratized N5E is a penetrating agent and it was able to destroy some of the dormant infections present in the nodal region of the stalks. As in other experiments, infection could be traced back to the bud and leaf scar. These results indicated that development of control measures based on the use of a seed treatment

might prove ineffective in preventing development of the fungus in the stalk during the winter months.

These studies indicated that the occurrence of the red rot organism in standing cane depended on the resistance of the cane to the disease. Isolation tests showed that there was a correlation between resistance and the number of isolates cultured from the leaf scars, buds and bud scales. The organism was isolated from a high percentage of the nodes of four susceptible varieties tested. With two out of three resistant varieties, the isolation of the organism was not frequent. However, the other resistant variety gave a rather high number of isolates from the leaf scars and bud scales. There was also a correlation between resistance and development of the disease in stalks placed in storage.

The number of isolations made from cane collected from the sugarcane area of Louisiana agreed closely with those obtained from the Louisiana State University experimental plots. Dusting with Cryolite to control the sugarcane borer, did not affect the number of cultures of the red rot fungus obtained from nodes of Co. 290 cane.

Studies on the occurrence of the conidial stage of the red rot fungus on green leaf sheaths of different varieties have shown that the number of leaf sheaths infected was not an indication of disease susceptibility. An examination of leaf sheaths and leaves of Co. 290 cane from the sugarcane area of Louisiana showed that conidial and perithecial stages were present on a high percentage of the sheaths and leaves examined.

Preliminary studies of the effect of environment on development of red rot in the sugarcane stalk indicated that dry conditions favored the development of the disease in stalks of Co. 290 cane. Treatments in which moist conditions prevailed apparently prevented development of the disease, since

the organism was found spreading in only a few nodes. Variations in temperature had no effect on the progress of the disease in the stalk. These results indicated that deterioration of seed pieces of certain varieties, which usually occurs during a winter of low temperature and wet weather, might not be due to red rot, but to Phytophthora. Phytophthora was repeatedly isolated from deteriorated stalks of many varieties (41). It was also shown by inoculation tests that the fungus was capable of causing a deterioration of the seed pieces similar to the one observed under field conditions.

Studies already discussed indicated that treatment of seed pieces with a fungicide before planting would require a fungicide of some penetrating ability. One of these studies demonstrated that the fungus could be greatly reduced by removing the leaf sheaths before they became infected. Control measures, such as the use of a fungicidal dust on seed plots during the growing season, might prevent infection of the sheaths as they pulled away from the stalk, thus preventing infection of the nodes which have been shown to be the point of entry of the fungus into the stalk. In order to test this hypothesis, dusting experiments were conducted over a two-year period. Results of isolation tests and studies of the development of the disease in stalks placed in storage have shown that the use of a dust on seed plots during the growing season reduced the number of infected nodes of variety Co. 290 by two-thirds. Parzate was found to be more effective than either Fermate or Zerlate.

Cane which had been dusted was used in planting tests conducted in the fall of the year. Stand count and yield data taken from tests conducted at Louisiana State University failed to show any significant increases over the non-dusted cane. The failure of the dusted cane to give an

increase was probably due to Phytophthora. By isolation tests, it was demonstrated that Phytophthora was present in red rot free stalks of Co. 290 cane grown in the greenhouse. Dusted cane plus a seed treatment before planting gave increases in stand and yield. For example, the treatment Parzate plus Teresan, gave a 256.0 per cent increase in stand and a 102.0 per cent increase in tonnage over the non-dusted check cane. Stand counts of cane which received only the dust treatment, taken from planting tests at Rillesud and Albania plantations, gave increases of 36.0 and 27.0 per cent respectively.

Table XX shows that stands of Co. 290 cane might be increased by dusting the cane to be used for seed with either Cryolite alone, Cryolite in combination with Parzate or with Parzate alone. The table also shows that soil type and date of planting might affect the stand in the spring. A comparison of the treatments at individual locations showed that the treatments Cryolite only and Cryolite plus Parzate, planted early on light soil, gave significant increases in shoot count. The Parzate treatment failed to give an increase in both the early and late plantings on light soil. Dugas (17) reported that canes dusted with Parzate gave higher borer counts than cane dusted with either Cryolite or a combination of Cryolite and Parzate. This borer increase also meant an increase in the number of holes made in the stalk through which the red rot fungus could gain entry into the sugarcane stalk. Use of this cane for seed purposes could account for the results obtained with Parzate in an area where borers were a problem. No significant increases were obtained between the different treatments in the late planting test on light soil. The early and late plantings on heavy soil showed significant differences between treatments.

Results of these studies indicated that Co. 290 cane should be planted early, especially on light soil. Early and late planting tests on light soil showed that significant increases in shoot count could be obtained when the cane was planted early in the fall. Early and late planting tests on heavy soil failed to show as great a difference between treatments as the light soil tests. A comparison of soil types and dates of planting, as shown in Table XX, showed that it might be important to consider the type of soil and date of planting when planting a susceptible variety like Co. 290.

The studies on red rot control indicate that further knowledge is needed as to the relative importance of the red rot fungus and Phytophthora with respect to stand failures of sugarcane in Louisiana. Red rot free cane when planted has failed to produce stands in the spring, indicating that Phytophthora rot or some other factor is responsible for some of the stand failures occurring in Louisiana.

SUMMARY

Studies on the methods by which the red rot fungus infects the sugarcane stalk were made using both resistant and susceptible varieties of cane.

After soaking apparently healthy stalks of variety Co. 290 cane for 24 hours in a solution of bichloride of mercury, the red rot fungus was cultured from the leaf scars, buds and bud scales.

Use of various fungicides to treat the cane before storage did not prevent development of the disease in the stalk.

A correlation was observed between resistance to the disease, isolation of the organism from the nodal region and development of the disease in stalks placed in storage.

Stalks of different varieties placed under unfavorable conditions developed red rot. Infection occurred at the nodal region. From infected nodes the infection could be traced back to the bud and leaf scar as the initial points of infection.

Removal of the leaf sheaths from the stalks of Co. 290 cane before they became infected greatly reduced the amount of latent infection in the nodal region.

Dry conditions favored development of the red rot fungus in stalks of Co. 290 cane, while moist conditions prevented spread of the disease in the stalk.

Infection of the leaf sheaths of different varieties by the fungus showed no correlation with disease susceptibility. The conidial and perithecial stages of the organism occurred on a high percentage of mature leaf

sheaths and leaves of variety Co. 290.

Appressoria formed and infection threads penetrated the epidermal cell wall of young bud scales 33 hours after inoculation with a conidial suspension of the red rot fungus. A cap or overgrowth formed around the infection thread in two resistant varieties and a susceptible variety studied. The cap was observed only in areas of thick-walled cells. In areas of thin-walled epidermal cells the fungus mycelium was found in the subepidermal cells. With a susceptible and a resistant variety studied, a dark gummy material often filled the intercellular spaces in advance of the mycelium. This material was produced in lesser amounts in the susceptible variety.

Healthy shoots attached to the seed piece developed the disease in storage. Infection occurred at the base of the shoot at the point of contact with the old bud scale.

A fungicidal dust applied on the cane during the growing season reduced the number of isolates which could be cultured from the nodal region and also reduced infection of the node in storage.

Planting tests in which dusted cane was used for seed failed to give consistent results. Phytophthora, which was shown to be pathogenic, caused a reduction in stand in these tests. However, similar tests on two plantations gave significant increases in shoot count in the spring. Increases in stand and yield resulted from the use of dusted cane and a seed treatment before planting.

In an area where the borer was a problem, stands of Co. 290 cane were improved by dusting the cane to be used for seed with either Cryolite or a combination of Cryolite and Parzate. Parzate-dusted cane planted on heavy soil gave significant increases in shoot count in the spring.

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AUTOBIOGRAPHY

René John Staib was born on July 28, 1918 at Vacherie, Louisiana. He was graduated from the St. James High School in May, 1936, and entered the Louisiana State University in September, 1936. In June, 1941, he received the Bachelor of Science degree in Vocational Agriculture from that Institution. In July, 1941, he entered the U. S. Army and served as an artillery officer until released from the Service in November, 1945. He re-entered the Louisiana State University in February, 1946, and received his Master of Science degree in August, 1947. He is now a candidate for the degree of Doctor of Philosophy in August, 1949.

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LEGEND FOR PLATES

PLATE I

Fig. 1. Nodes from Co. 290 treated for 30 minutes in 1-1000 bichloride of mercury in 50 per cent alcohol, then stored for approximately one month at 70°F. with the sterilizing agent left on. Bud type infection occurred in these three nodes. Note the destruction of the bud by the fungus. In the right node the fungus is spreading into the internode.

Fig. 2. Nodes of Co. 290 treated and stored as in Fig. 1. Leaf scar type infection occurred in these three nodes. In the node on the left note the spread of infection from the initial point of entry. The two other nodes show spread of infection into the adjoining internode.

PLATE II

Fig. 3. Co. 290 cane planted in sand and dug when shoots were 3 or 4 inches tall. After digging, cane stored for 2 weeks at 70°F. with shoots adhering to the stalks. Infection of shoot by red rot fungus at the point of contact with the old bud scale. Three nodes show spread of the fungus inward from the initial point of infection.

Fig. 4. Nodes of Co. 290 planted and stored under same conditions as nodes in Fig. 3. Stalks split longitudinally to show spread of the red rot fungus from the initial point of infection, the old bud scale. Note the spread up into the shoot and also spread into the mother stalk.

PLATE III

Fig. 5. Free hand sections made after young bud scale of Co. 290 grown in the greenhouse were inoculated with a spore suspension of the red rot fungus. Appressoria formed and infection threads penetrated the epidermal cell wall 30 hours after inoculation. Note the two infection threads and cap or overgrowth around the pegs after entry into the lumen of the cell (x 440).

Fig. 6. Free hand sections made after young bud scales of C.P. 34/120 grown in the greenhouse were inoculated with a spore suspension of the red rot fungus. Sections made 33 hours after inoculation. Note the same infection and overgrowth as in Fig. 5. (x 440).

Fig. 7. Bud scale of Co. 290 grown in the greenhouse sectioned (free hand) 77 hours after inoculation with a spore suspension of the organism. This shows the infection thread and enlargement of the cap around the thread as it penetrated deeper into the cell (x 950).

Fig. 8. Free hand section of young bud scale of a resistant variety (C.P. 36/13) made 50 hours after inoculation with a conidial suspension of the fungus. Note with this variety that a fine thread and apparently the same overgrowth were found in the subepidermal cells (x 440).

PLATE IV

Fig. 9. Bud scale of variety C.P. 34/120 inoculated with spores of the red rot fungus and sectioned 33 hours later. Note that entry of the fungus into the subepidermal cells of the bud scale occurred in an area of thin-walled epidermal cells (x 950).

Fig. 10. Enlarged photograph of Fig. 9 showing areas of thick-walled cells and presence of a dark material in the intercellular spaces below the point of infection (x 440).

PLATE I

Fig. 1

Fig. 2

PLATE II

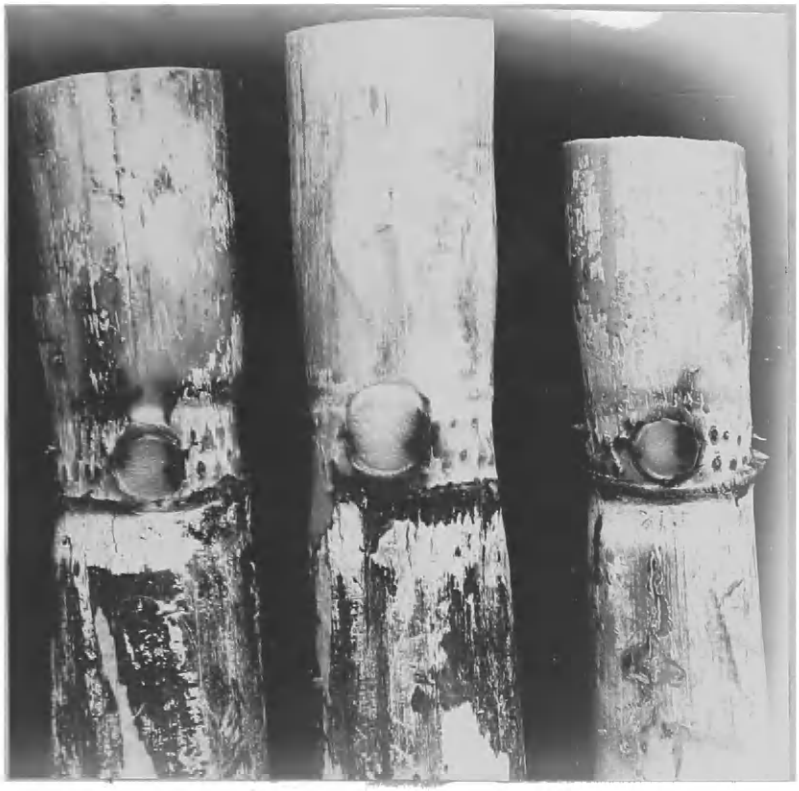

Fig. 3.

Fig. 4

PLATE XIII

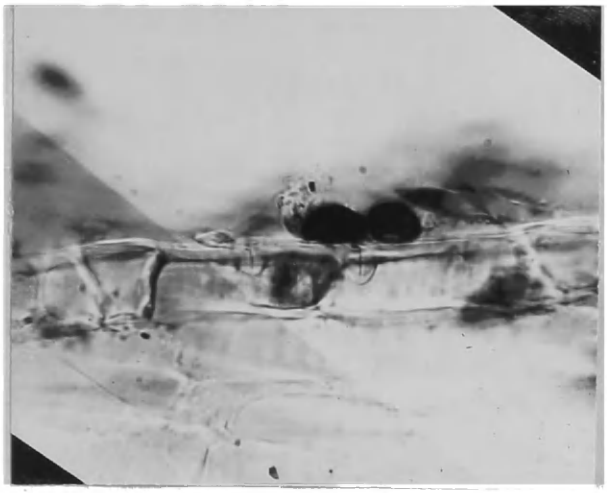

Fig. 5

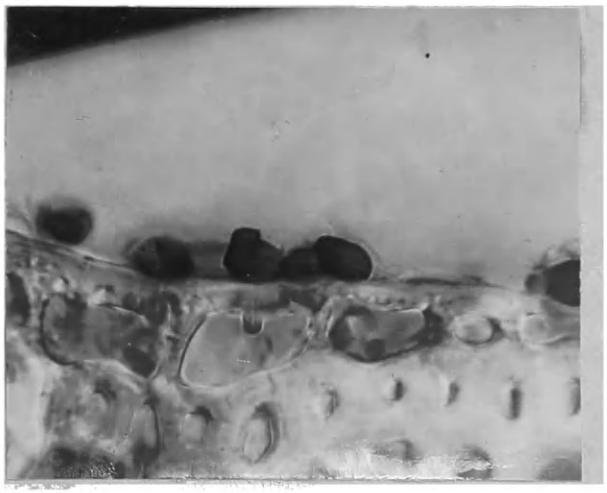

Fig. 6

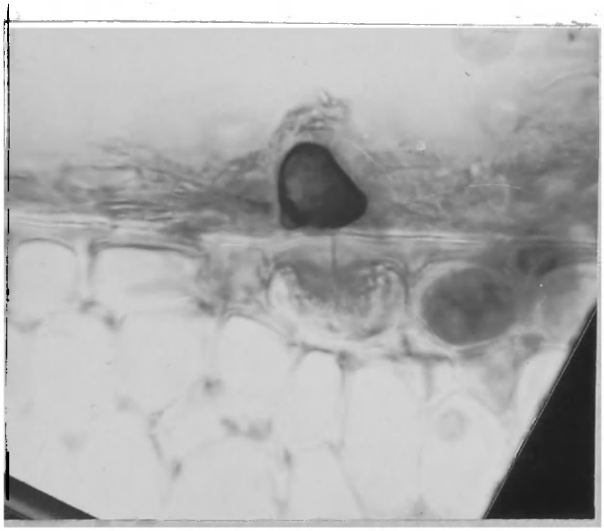

Fig. 7

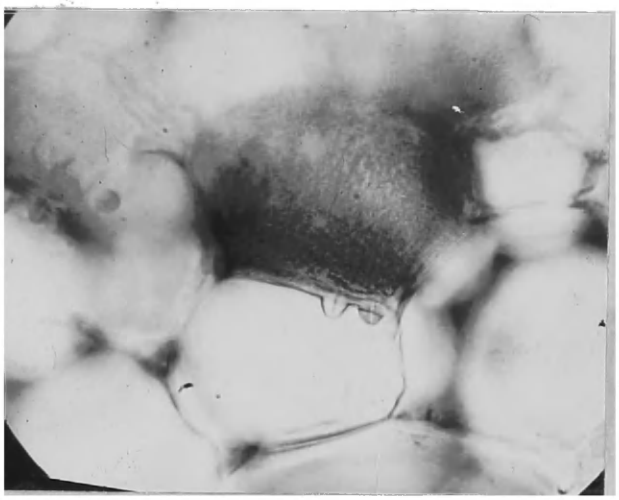

Fig. 8

PLATE IV

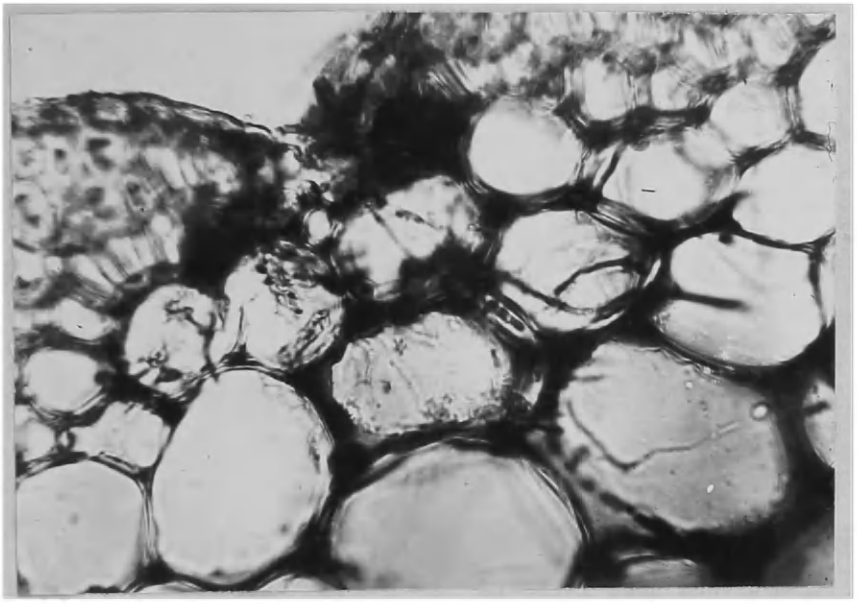

Fig. 9

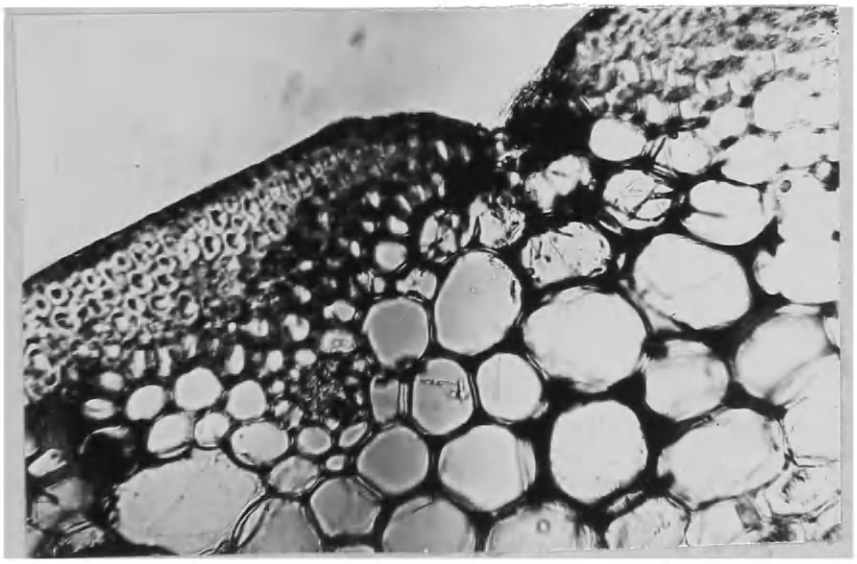

Fig. 10

EXAMINATION AND THESIS REPORT

Candidate: René J. Steib

Major Field: Plant Pathology and Agronomy

Title of Thesis: STUDIES ON METHOD OF INFECTION AND CONTROL OF THE RED ROT FUNGUS OF SUGARCANE

Approved:

C W Edgerton

Major Professor and Chairman

Richard J Russell

Dean of the Graduate School

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Date of Examination:

August 4, 1949